

The University of Chicago

STUDIES OF BROMIDES
AND CHLORIDES IN BLOOD AND
CEREBROSPINAL FLUID

- I. THE EFFECT OF CHANGING THE ACID-BASE BALANCE ON CON-
CENTRATION AND DISTRIBUTION OF BROMIDES AND
CHLORIDES IN THE BLOOD OF DOGS
- II. CONCENTRATION AND DISTRIBUTION OF BROMIDES AND
CHLORIDES IN THE BLOOD AND CEREBROSPINAL
FLUID OF HUMAN SUBJECTS

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF MEDICINE

1932

By

MEYER SAMUEL MISHKIS

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PART I. INTRODUCTION

This study consists of two parts. The first part is a study of the effect of changing the acid-base balance on the concentration and distribution of bromides and chlorides in the blood of dogs. The second part consists of a study of the distribution of bromides in the serum, blood cells and cerebrospinal fluid of humans.

Numerous authors during the last half century have studied the distribution of naturally occurring salts, water and gases between the cells and serum in the blood. L. J. Henderson (1) has correlated the findings of these authors and shown that a change in any one of the constituents mentioned affects all the others. Van Slyke, Wu and McLean (5) have shown that bicarbonates and chlorides are distributed between the cells and serum in vitro in a manner consistent with that predicted by the Gibbs-Donnan Distribution Law. Several apparent exceptions to this law have been observed. First, Van Slyke, Hastings et al. observed that the ratio $\frac{(\text{HCO}_3)_c}{(\text{HCO}_3)_s}$ and the ratio $\frac{(\text{Cl})_c}{(\text{Cl})_s}$ were not identical even in vitro. Then followed Peters' observation (6) that chloride distributions were encountered clinically which did not follow the predictions of the Donnan Law. Finally, the similarity between the behavior of bromides and chlorides led Hastings and van Dyke (2, 8) and Hastings, Harkins and Liu (3, 7) to test the applicability of the Donnan Law to the distribution of bromide ions. They found that bromides showed an even greater discrepancy than chlorides and bicarbonates in their distribution between cells and serum both in vitro and particularly in vivo.

The above observations led to this study to determine some of the factors which might be responsible for the discrepancy between the observed distribution of bromides and chlorides between cells and serum and that predicted by the Donnan Law. One of the main purposes of this study was to determine whether changes in pH produced in vivo would result in a distribution of bromides which might be predicted by this law.

METHOD OF PROCEDURE

In attacking the problems outlined in the introduction experiments were devised whereby the following conditions could be studied both in the normal animal and during alkalosis and acidosis:

1. Bromide concentration in cells and serum.
2. Chloride concentration in cells and serum.
3. Total halide concentration in cells and serum.
4. Percentage of total halide present as bromide in cells and serum.
5. Distribution of total halide between cells and serum.
6. Distribution of bromide between cells and serum.
7. Distribution of chloride between cells and serum.

Experimental Procedure

Normal pH.- Large dogs were given 242 millimols of sodium bromide by mouth in 250 cubic centimeters of water followed by 150 cubic centimeters of additional water. When exceptions to this procedure occurred they will be noted in the tables. Samples of blood were drawn before and at frequent intervals during eight to sixteen days after the administration. Bromide and chloride concentration and percentage of water of the cells and serum of these samples were determined. These experiments will be referred to as the normal-bromide experiments.

Alkalosis.- After a period of two to five months had elapsed since the last administration of sodium bromide to allow for complete excretion of the bromide, alkalosis was produced in the same animals by the oral administration of 25 grams of sodium bicarbonate in a 5 per cent solution twice daily for a period of three to five days preceding the administration of a new dose of sodium bromide. The administration of sodium bicarbonate was continued during the period that blood samples were taken. The serum pH and the bromide, chloride and water content of all samples of cells and serum were determined. These experiments will be referred to as the alkalosis-bromide experiments.

Acidosis.- After a similar period of two to five months as described in the preceding paragraph, acidosis was produced in the same animals by the oral administration of 10 grams of ammonium

chloride in a 2.5 per cent solution twice daily for a period of three to five days preceding the administration of sodium bromide and continued throughout the period that blood samples were taken. The same determinations were made as in the alkalosis-bromide experiments. These experiments will be referred to as the acidosis-bromide experiments.

In all, twenty-five experiments on eighteen dogs were attempted. Due to uncontrollable difficulties, however, only eleven experiments on six dogs were brought to completion. Of these, four were on normal dogs, five during alkalosis and two during acidosis. In this part the details of four normal-bromide experiments, two alkalosis-bromide experiments and two acidosis-bromide experiments will be reported and discussed.

Analytical Methods

Blood for analysis was drawn from the jugular vein directly into a centrifuge tube under oil. It was then defibrinated and centrifuged. The cells and serum were separated and samples were taken immediately. All cell analyses were made on weighed amounts of cells; all serum analyses were made on accurately measured volumes of serum. The pH of the serum was determined colorimetrically by the method of Hastings and Sendroy (4). Water determinations on cells and serum were obtained by drying a known weight of cells and a known volume of serum to constant weight at 110°. Bromide and chloride determinations were made by electrometric titration of protein free filtrates (2).

Calculations and Abbreviations

All values of bromide and chloride concentration are expressed in terms of millimols per kilogram of water of cells or serum. The water content is expressed as grams of water per gram of cells and as grams of water per cubic centimeter of serum.

Upon standardization of the method for the electrometric titration of bromide in cells it was found that a correction greater than that found by Hastings and van Dyke had to be applied. The correction factors in cubic centimeters are given in Figure 1. For example, if the point of inflection occurred at 1.50 cubic centimeters, the corrected end point should be 1.50-0.17 or 1.33 cubic centimeters.

The following abbreviations have been used:

mM denotes millimols.

(Br)_c and (Br)_s denote concentration of bromide in milli-

mols per kilogram of cell or serum water.

$(Cl)_c$ and $(Cl)_s$ denote concentration of chloride in millimols per kilogram of cell or serum water.

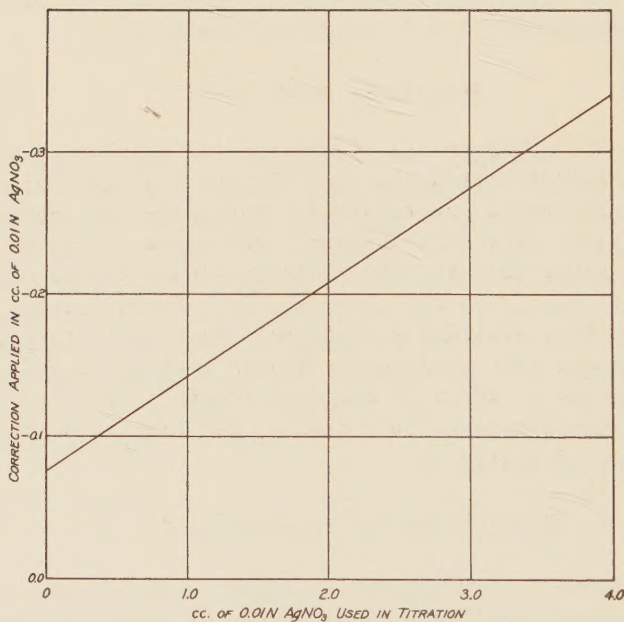


FIG. 1 ELECTROMETRIC TITRATION CORRECTIONS FOR BROMIDES IN CELLS

EXPERIMENTAL RESULTS

The experimental results of the seven series of observations outlined in the methods of procedure are given in detail in the Appendix.* Tables I to IV include the results of four normal-bromide experiments. Tables V and VI include the results of two alkalosis-bromide experiments. Tables VII and VIII include the results of two acidosis-bromide experiments. Table IX is based on the data presented in the previous tables. The figures presented in this section are based on these tables.

Bromide Concentrations in Cells and Serum

Normal pH.- The concentrations of bromide in the cells and serum of the normal animals are plotted against time in Figure 2. These curves show that the absorption of bromide into the blood stream was rapid, concentrations of 5 and 16 millimols per kilogram of serum water being found in dogs, Be and Sm, respectively, fifteen minutes after the administration of the sodium bromide. However, the maximum serum bromide concentration recorded was not reached until one to five hours after the bromide was given. The serum bromide concentration decreased quite slowly. Indeed, in three of the dogs there was not much change during the first forty-eight hours. (In dog, Sm, the data are not sufficiently complete to permit a statement on this point.) Subsequent observations up to a maximum of ten days showed a progressive but slow decrease in serum bromide.

The cell bromide concentrations increased initially more rapidly in two of the dogs than did those of the serum. The subsequent changes, however, were approximately parallel to the serum bromide changes.

Analyses made thirty-three days after the single dose of bromide had been given revealed no measurable amount of bromide in the serum, but some was still present in the cells in a concentration of 5 millimols per kilogram of cell water. To make more sure the complete elimination of bromide after a previous experiment two to five months were allowed to elapse when initial bromide tests were made before new experiments were started on the same dog. At this time bromide was absent from both the serum and the cells.

Alkalosis.- The effect of the administration of sodium bi-

*The Appendix is to be found only in the complete dissertation, on file in the University of Chicago Libraries.

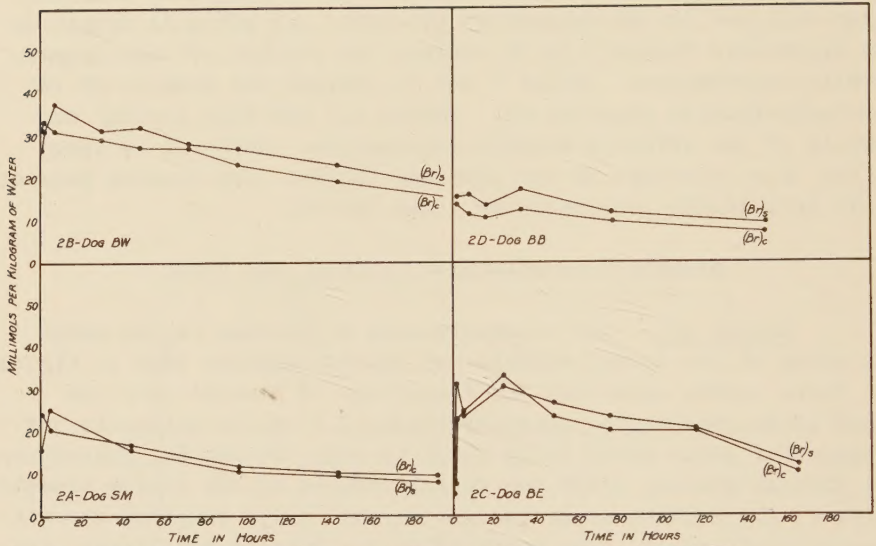


FIG. 2 THE CHANGE WITH TIME IN THE BROMIDE CONCENTRATION OF THE CELLS AND SERUM OF NORMAL DOGS

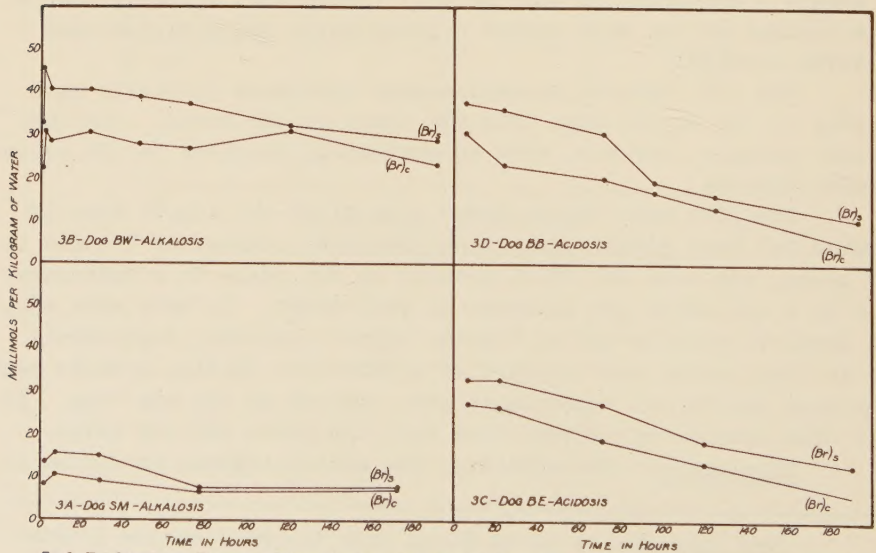


FIG. 3 THE CHANGE WITH TIME IN THE BROMIDE CONCENTRATION OF THE CELLS AND SERUM OF DOGS BLOOD IN ALKALOSIS AND IN ACIDOSIS

carbonate prior to the administration of bromide and for the entire duration of the experiment on the cell and serum bromide concentrations is illustrated in Figure 3, A and B. Inspection of these curves does not reveal a striking difference between the alkalosis-bromide and the normal-bromide experiments on the same animals. There is an indication that the presence of the alkalosis slowed the rate of decrease in cell and serum bromide concentration. This is more clearly brought out by plotting the data as in Figure 4. In these figures the bromide concentration at the end of the first twenty-four hour period is chosen as the concentration of reference and called 100. Subsequent concentrations at twenty-four hour intervals are expressed as percentages of this concentration. The unshaded areas in the blocks represent the percentage of bromide present in the cells and serum at normal pH and the shaded areas the percentages present in alkalosis. Viewed in this way, it is seen that one week after the bromide was given, there still remained 10 and 13 per cent more bromide in the serum and 18 and 29 per cent more bromide in the cells in the alkalosis than in the normal experiments.

Acidosis.- The effect of the administration of ammonium chloride prior to the administration of bromide and for the entire duration of the experiment on the cell and serum bromides is illustrated in Figure 3, C and D. Inspection of these curves does not reveal a striking difference between acidosis-bromide and the normal-bromide experiments. The alkalosis effects were similarly masked until the concentrations were referred to the initial twenty-four hour concentration. When acidosis effects are studied in the same way as illustrated in Figure 5 it is seen that the administration of ammonium chloride hastened the rate of decrease in cell and serum bromide concentration.

Chloride Concentration in Cells and Serum

Normal pH.- The concentrations of chloride in the cells and serum of the normal animals are plotted against time in Figure 6. These curves show that the changes in cell and serum chloride were, in general, opposite to the bromide changes. There was a rapid drop in serum chloride within the first two hours followed by a gradual return toward normal, which, however, was not complete even at the end of ten days. In two of the dogs, BB and Be, the serum chloride was still 25 and 30 millimols, respectively, below its normal concentration forty hours after the bromide had been administered.

Alkalosis.- The effect of the administration of sodium

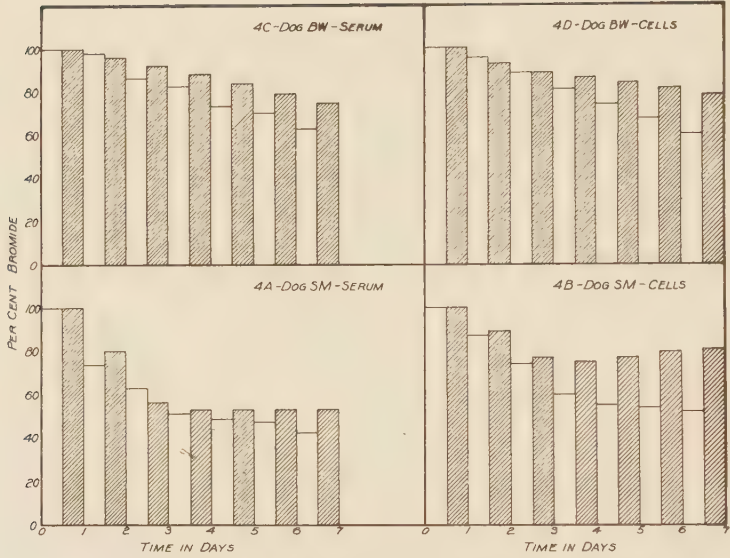


FIG. 4 THE CHANGE IN CELL AND SERUM BROMIDE EXPRESSED AS PER CENT OF THE CELL AND SERUM BROMIDE CONCENTRATION FOUND 24 HOURS AFTER ADMINISTRATION OF SODIUM BROMIDE. THE UNSHADED BLOCKS REPRESENT NORMAL BROMIDE EXPERIMENTS AND THE SHADED BLOCKS ALKALOSIS-BROMIDE EXPERIMENTS.

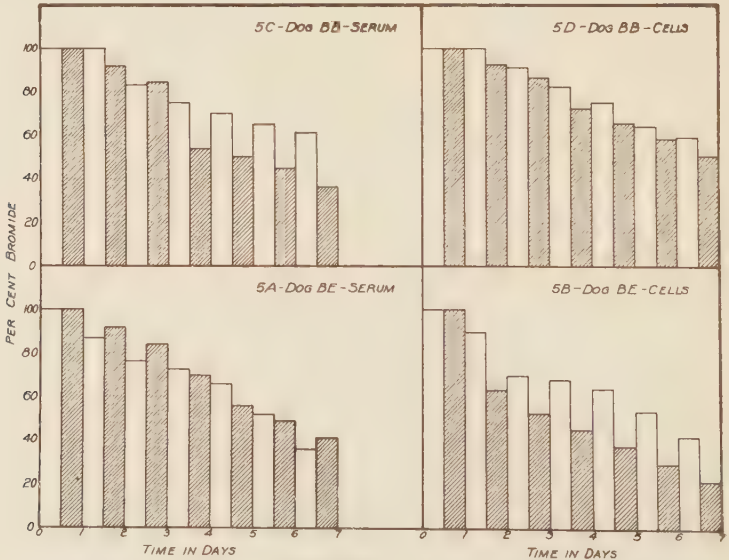


FIG. 5 THE CHANGE IN CELL AND SERUM BROMIDE EXPRESSED AS PER CENT OF CELL AND SERUM BROMIDE CONCENTRATION FOUND 24 HOURS AFTER ADMINISTERING SODIUM BROMIDE. THE UNSHADED BLOCKS REPRESENT NORMAL BROMIDE EXPERIMENTS AND THE SHADED BLOCKS ACIDOSIS-BROMIDE EXPERIMENTS.

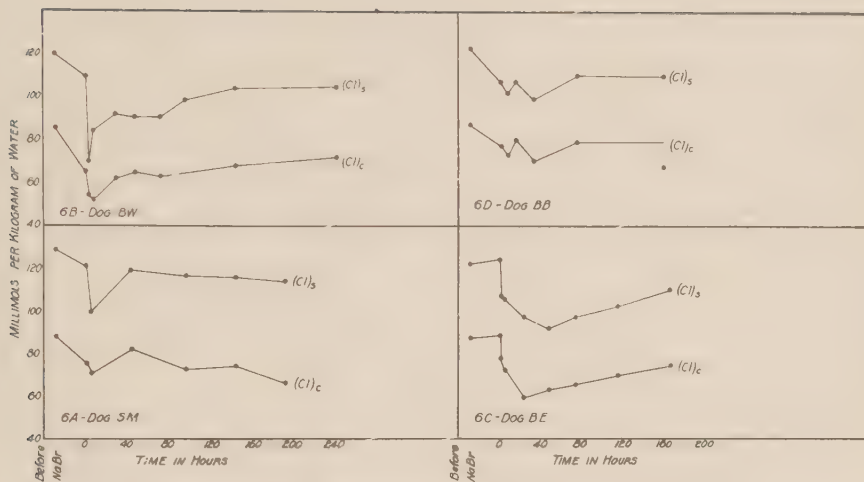


FIG. 6 THE CHANGE WITH TIME IN THE CHLORIDE CONCENTRATION OF THE CELLS AND SERUM OF NORMAL DOGS

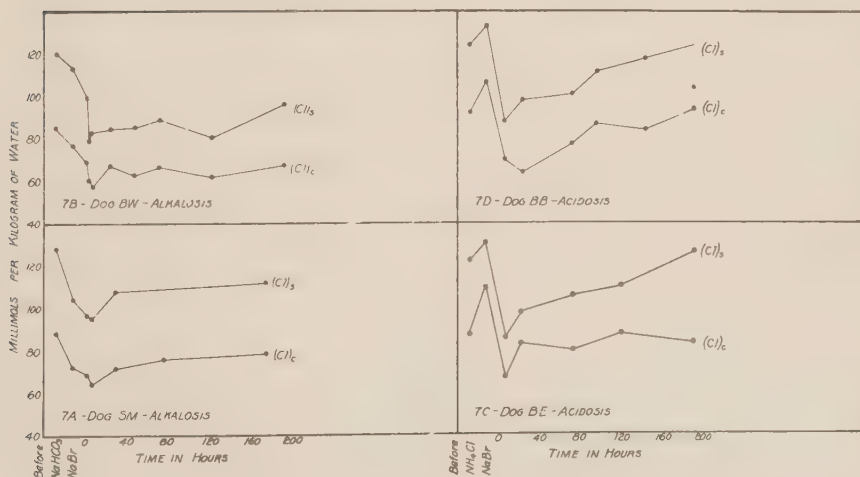


FIG. 7 THE CHANGE WITH TIME IN THE CHLORIDE CONCENTRATION OF THE CELLS AND SERUM OF DOGS IN ALKALOSIS AND IN ACIDOSIS

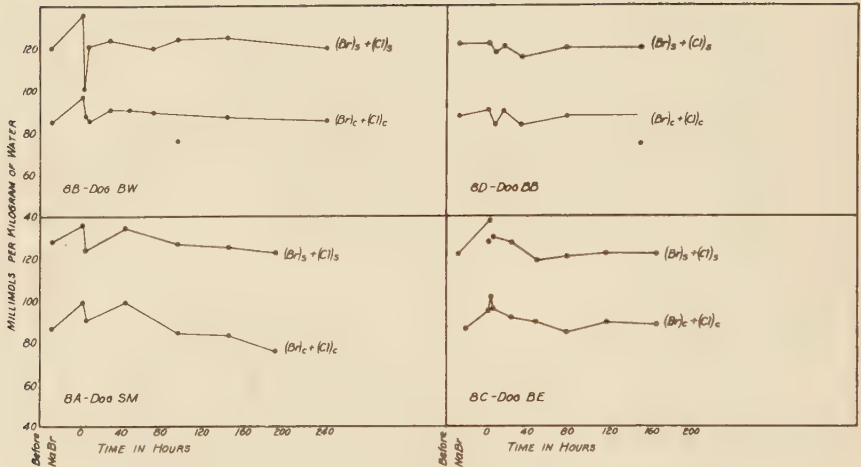


FIG. 8 THE CHANGE WITH TIME IN THE TOTAL HALIDE CONCENTRATION OF THE CELLS AND SERUM OF NORMAL DOGS

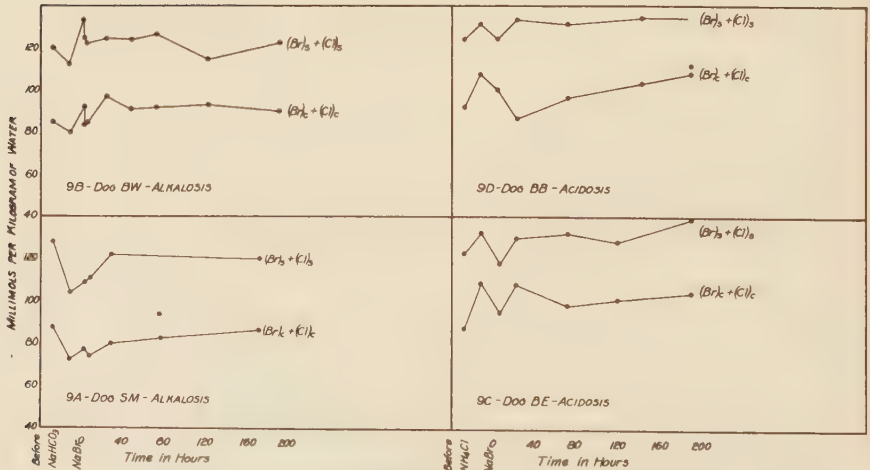


FIG. 9 THE CHANGE WITH TIME IN THE TOTAL HALIDE CONCENTRATION OF THE CELLS AND SERUM OF DOGS IN ALKALOSIS AND IN ACIDOSIS

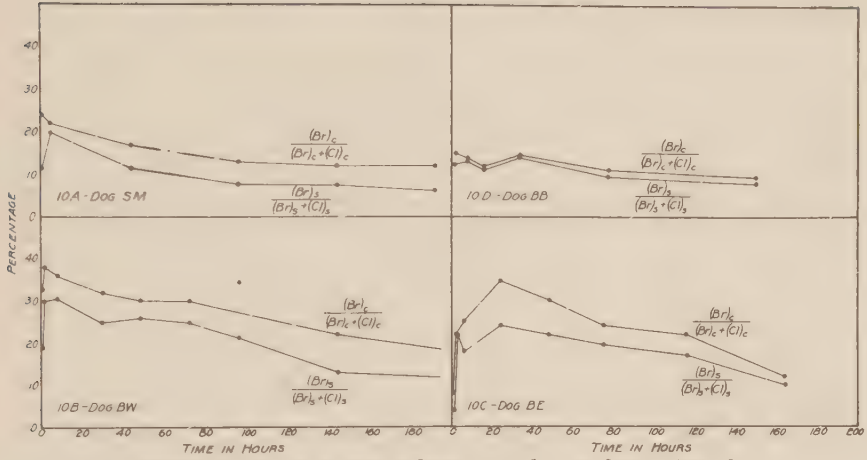


FIG 10 THE PERCENTAGE OF TOTAL HALIDE PRESENT AS BROMIDE IN THE CELLS AND SERUM OF NORMAL DOGS.

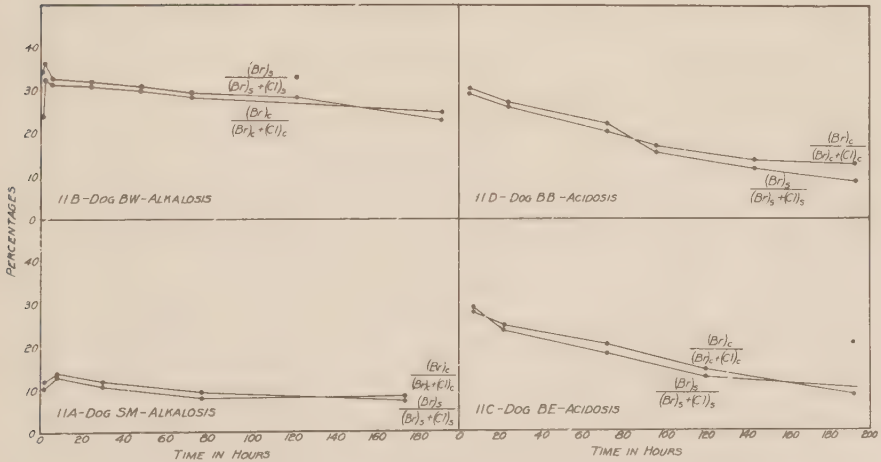


FIG. 11 THE PERCENTAGE OF TOTAL HALIDE PRESENT AS BROMIDE IN THE CELLS AND SERUM OF DOGS IN ALKALOSIS AND IN ACIDOSIS.

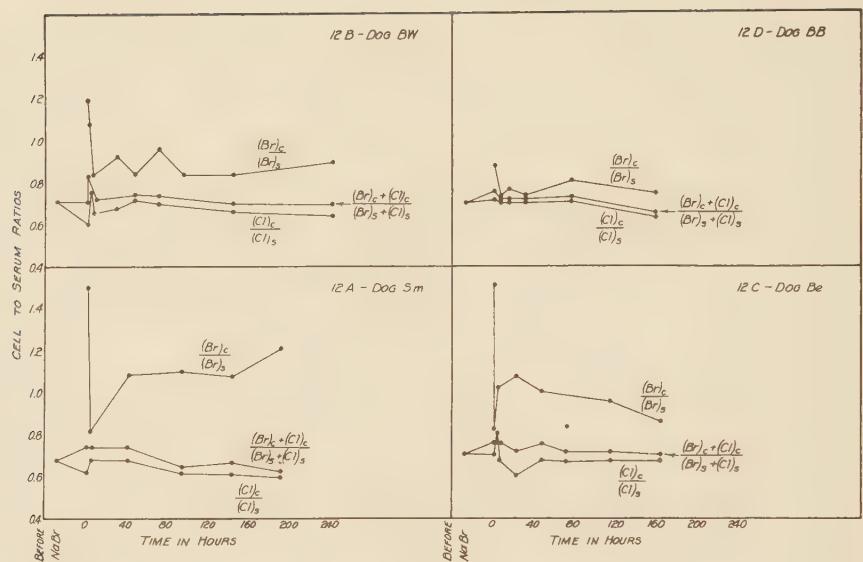


FIG.12 THE DISTRIBUTION OF BROMIDE, CHLORIDE AND TOTAL HALIDE IONS BETWEEN CELLS AND SERUM OF NORMAL DOGS.

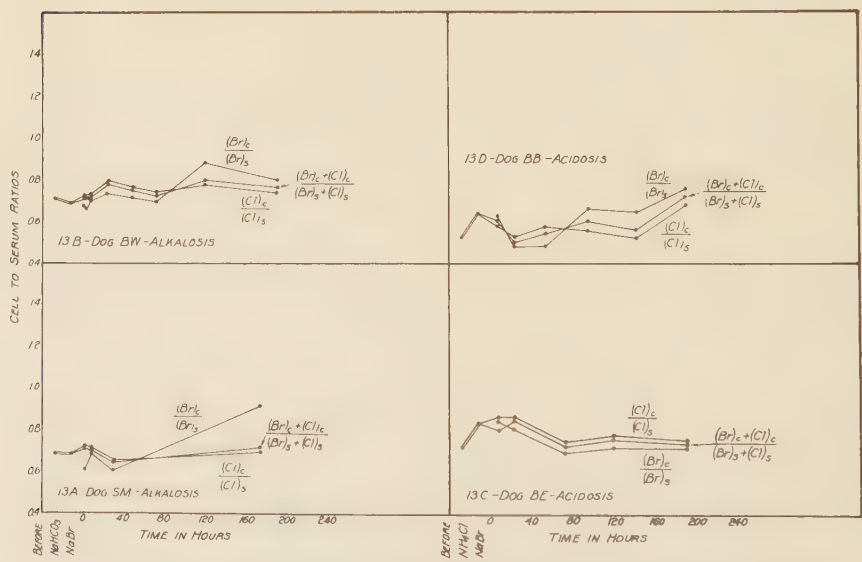


FIG.13 THE DISTRIBUTION OF BROMIDE, CHLORIDE AND TOTAL HALIDE IONS BETWEEN CELLS AND SERUM OF DOGS IN ALKALOSIS AND IN ACIDOSIS.

bicarbonate (as described in the preceding section) on the cell and serum chloride concentration is illustrated in Figure 7, A and B. These curves show that the administration of sodium bicarbonate did not materially alter the path of the return of the serum chloride toward normal.

Acidosis.- The effect of the administration of ammonium chloride (as described in the preceding section) on the cell and serum chloride concentrations is illustrated in Figure 7, C and D. These curves show that the initial decrease in serum chloride upon administration of sodium bromide was greater and its return to normal more rapid than in the normal-bromide experiments.

In each of the above three conditions of the blood the changes in cell chloride followed those observed in the serum.

Total Halide Concentration in Cells and Serum

Normal pH.- The concentrations of bromide plus chloride in the cells and serum of the normal animals are plotted against time in Figure 8. These curves show that in three of the four animals there was an increase in total serum halide concentration amounting to 8, 15 and 16 millimols within the first five hours after the bromide was given; in the fourth, no change occurred. A return to approximately the normal value occurred within the first twenty-four hours following which no change of significance was noted. That the initial increase in serum halide was not simply due to loss of water from the serum, but was a real gain of base and halides, is indicated by the fact that no concomitant concentration of the serum occurred as evidenced by the lack of change in the water content of the serum.

Alkalosis.- The effect of the administration of sodium bicarbonate (as described previously) on the bromide plus chloride concentration of cells and serum is illustrated in Figure 9, A and B. These curves show that the total serum halide remained for the duration of the experiment well above its value before sodium bromide was administered.

Acidosis.- The effect of the administration of ammonium chloride (as described previously) on the bromide plus chloride concentration of cells and serum is illustrated in Figure 9, C and D. These curves show that the administration of ammonium chloride, as might be expected, resulted in an increase of total serum halide, but that the subsequent administration of sodium bromide, contrary to expectation, resulted in a decrease of total serum halide. This decrease was temporary for after twenty-four hours the total serum halide returned to the abnormally high value

of 130 millimols per kilogram of water, and persisted at this level throughout the experiment.

In each of the above three conditions of the blood the changes in total cell halide paralleled those observed in the serum.

Percentage of Total Halide Ion Present as Bromide in Cells and Serum

Normal pH.- The percentage of total halide present as bromide in the cells and serum of the normal animals was plotted against time in Figure 10. These curves show that within five hours after giving sodium bromide the serum halide was between 20 and 30 per cent bromide in three of the dogs. (In one animal the serum halide was only 13 per cent bromide, but this may be attributed to the failure of this animal to absorb all of the bromide given.) The percentage of halide present as bromide in the cells was higher than that in the serum and persisted so throughout the experiment.

Alkalosis.- The effect of the administration of sodium bicarbonate (as described previously) on the percentage of total halide present as bromide in the cells and serum is illustrated in Figure 11, A and B. These curves show that when sodium bicarbonate was administered there was no essential difference between the percentage of halide present as bromide in the cells and in the serum. The effect of the bicarbonate in eliminating the difference between cells and serum noted in the normal-bromide experiments may be due either to a decrease in the percentage of halide present as bromide in the cells or to an increase in the percentage of bromide present in the serum.

These curves also show the delaying effect of sodium bicarbonate on bromide removal. For example, in dog, B & W, the percentage of serum halide present as bromide decreased from 30 per cent at the eighth hour to 13 per cent at the one-hundred and forty-fourth hour in the normal-bromide experiments, whereas it had only decreased from 33 per cent to 27 per cent over a comparable period of time in the alkalosis-bromide experiments.

Acidosis.- The effect of the administration of ammonium chloride (as described previously) on the percentage of total halide present as bromide in the cells and serum is illustrated in Figure 11, C and D. These curves like those in the alkalosis experiments also show no difference between the cell and serum bromide percentages.

These curves also show the accelerating effect of ammonium chloride on bromide removal. For example, in the normal-bromide

experiment on dog, Be, a decrease of 10 per cent occurred between the eighth and one-hundred and forty-fourth hour, but in the acidosis-bromide experiment a decrease of 16 per cent was noted during a comparable time interval.

Distribution of Total Halide between Cells and Serum

Whereas the preceding section dealt with the ratio of bromide to total halide in the cells and in the serum, this and the two subsequent sections will deal with the ratios of total halide, bromide and chloride concentrations in the cells to those in the serum.

Normal pH.- The total halide distribution between cells and serum in the normal animals is plotted against time in Figure 12. These curves show that the total halide distribution ratio rose slightly but consistently soon after giving the bromide without significant change in serum pH. Such an increase would be expected from the fact that the administration of bromide causes an increase in serum base which is not diffusible into the cells. The mathematical interpretation of this fact is found in equation 10 of the Van Slyke, Wu and McLean paper which is:

$$r = \frac{[A^-]_c}{[A^-]_s} = 1 - \frac{[BP]_c + [Hb]_c - [BE]_s}{2 ([B]_s - [BP]_s)}$$

An increase of $[B]_s$ (the total base of serum) would result in an increase in the distribution ratio, r , which would be accompanied by an increase in the total halide distribution ratio.

Alkalosis and Acidosis.- The total halide distribution between cells and serum in alkalosis and in acidosis is plotted against time in Figure 13. These curves do not show a striking difference between the total halide ratio in the normal condition and in alkalosis and acidosis. If, however, the total halide ratios are plotted against pH's as in Figure 14 A the effect of pH becomes more apparent. The solid line in the figure shows the distribution ratios predicted by the Van Slyke, Wu and McLean equation. Although there is scattering of the ratio points on both sides of the line, the ratios show a tendency to vary with the pH in the anticipated manner. (It may be noted in passing that the scattering is not as great as that observed by Hastings, Sendroy, McIntosh and Van Slyke (9) in a series of observations on the distribution of chloride and bicarbonate in a number of normal and pathological human subjects.) It may be concluded from this that the administration of sodium bromide, sodium bicarbonate or ammonium chloride does not result in changes in total halide distribu-

tions which are inconsistent with the predicted changes.

Distribution of Bromide between Cells and Serum

Normal pH.- The bromide distribution between cells and serum in the normal animals is plotted against time in Figure 12. These curves show a ratio of bromide concentration in cells to that in serum considerably and persistently higher than the total halide cell to serum ratio.

Alkalosis and Acidosis.- The bromide distribution between cells and serum in alkalosis and in acidosis is plotted against time in Figure 12. These curves show a ratio of bromide concentration in cells to that in serum considerably and persistently higher than the total halide cell to serum ratio.

If one plots the bromide cells to serum ratios against pH's, as in Figure 14 B, it may be seen that the points corresponding to the bromide cells to serum ratios at normal pH arrange themselves much above the line which would mark their location as predicted by the Van Slyke, Wu and McLean equation. Both at lower pH's and more especially at higher pH's these points distribute themselves more closely about the predicted line. From this it may be concluded that pH is an important factor in determining the distribution of bromide between cells and serum and that at normal pH this distribution is inconsistent with the Gibbs-Donnan Distribution Law.

Distribution of Chloride between Cells and Serum

Normal pH.- The chloride distribution between cells and serum in the normal animals is plotted against time in Figure 12. These curves show that the chloride cells to serum ratios parallel the total halide ratios but at a lower level. The difference between the two distribution levels is due not to a deviation in the chloride distribution of itself, but is dependent upon the higher-bromide ratios.

Alkalosis and Acidosis.- The chloride distribution between cells and serum in alkalosis and in acidosis is plotted against time in Figure 13. These curves do not show any striking difference between the chloride cells to serum ratios in the normal condition and in alkalosis and acidosis. If, however, the chloride ratios are plotted against pH's as in Figure 14 C the effect of pH becomes more apparent. It may be seen that the points corres-

ponding to the chloride cell to serum ratios at normal pH arrange themselves much below the line which would mark their location as predicted by the Van Slyke, Wu and McLean equation. As in the case of bromide ion, both at lower pH's and more especially at higher pH's these points distribute themselves more closely about the predicted line.

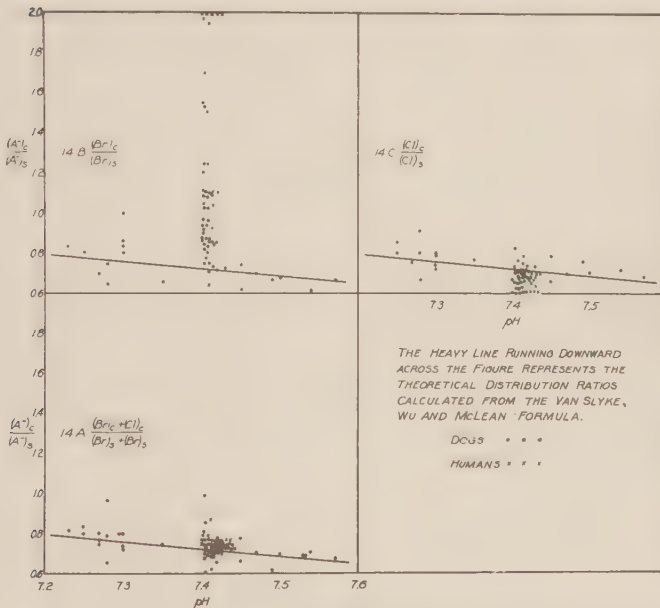


FIG. 14 THE CHANGE WITH pH OF THE BROMIDE, CHLORIDE AND TOTAL HALIDE CELLS TO SERUM DISTRIBUTION RATIOS IN DOGS AND IN HUMAN SUBJECTS.

DISCUSSION AND CONCLUSIONS

Summary of Results

Any interpretation of the foregoing results must be consistent with the following summary of the most important points of these results:

1. Absorption of bromide was rapid, its elimination slow. Alkalosis retarded, acidosis hastened the elimination. The absorption appeared to be faster, the elimination slower and the fluctuation less in the cells than in the serum.

2. On administration of sodium bromide the chloride concentration of cells and serum decreased more but returned faster to its previous value in acidosis than in the normal condition.

3. On administration of sodium bromide the total halide concentration of cells and serum of the normal animal increased slightly and temporarily but rapidly returned to the normal value; in alkalosis it rose slightly and continued at this higher level; in acidosis it decreased temporarily but returned to a higher level than the normal.

4. The per cent of total halide present as bromide was greater in the cells than in the serum of the normal animal, but this difference disappeared in alkalosis and in acidosis.

5. On the administration of sodium bromide the total halide ratio between cells and serum was slightly increased but this distribution ratio in the normal condition and in alkalosis and in acidosis was consistent with the Gibbs-Donnan Distribution Law.

6. On the administration of sodium bromide to the normal animal the bromide cells to serum ratio was much higher than that predicted by the Gibbs-Donnan Distribution Law, but in alkalosis and in acidosis the ratio accorded with this law.

7. On the administration of sodium bromide to the normal animal the chloride cells to serum ratio was lower than that predicted by the Donnan Law, but in alkalosis and acidosis the chloride ratio, like the bromide ratio, accorded with this law.

Discussion of Results

The points enumerated in the preceding section will be discussed under the following headings: (a) Change in Concentration

of Halides with Time; (b) Distribution of Halides between Cells and Serum.

(a) Change in Concentration of Halides with Time

The total halide concentration of the cells and serum was maintained at approximately the same level throughout all the experiments. The addition of bromide ion to the already present chloride ion had a displacing rather than additive effect on the total halide concentration. Changes in the chloride concentration with time were entirely due to changes in bromide concentration. Changes in the bromide concentration with time depended on both excretion from and absorption into the blood stream.

Of the total quantity of bromide administered (242 mM and in one case 388 mM) not more than one third appeared in the blood stream at any one time. Appelmans (10) found from five to ten times as much bromide in portions of the intestinal mucosa as in the blood thirteen hours after bromide administration. Absorption into the blood must, therefore, not be confined only to the first few hours following the administration of the bromide. The decrease in bromide concentration in cells and serum with time is a function, therefore, not only of the rate of excretion of bromide, but also of the rate of its absorption from the gut and other tissues into the blood.

Although the bromides promptly enter the cells after sodium bromide has been given, it appears that passage of bromide from cells to serum may not occur so readily since fluctuations in serum bromide concentrations are not always followed by similar fluctuations in the cell bromide, the latter decreasing much more regularly than the former.

(b) Distribution of Halides between
Cells and Serum

The distribution of total halides between cells and serum in all cases was such as could be predicted by the Gibbs-Donnan Law. The distribution of chloride alone between cells and serum was also found by Van Slyke, Wu and McLean to be such as could be predicted by this law. On the administration of bromide, however, the distribution of chloride in the normal animal was inconsistent with this law but the irregularity could be explained by the abnormal bromide distribution.

The distribution ratio of bromide between cells and serum of the normal animal in practically all cases was higher than the ratio predicted by the Gibbs-Donnan Law. Similar high distribution

ratios of bromide between cells and serum were also found by Hastings and van Dyke (2, 8) and by Hastings, Harkins and Liu (3, 7). In addition, the results of this study have shown that in both alkalosis and acidosis the bromide distribution ratio decreased.

A decrease of 0.05 in the bromide distribution ratio in the alkalosis-bromide experiments could be accounted for by the increase in pH observed but since a decrease in ratio of approximately 0.2 was found, this explanation is inadequate. If one adopts the hypothesis suggested by Hastings and van Dyke that at normal blood pH part of the cell bromide is present in combination with an unidentified cell constituent in non-ionic form it must be further assumed that such a combination is unstable at higher pH values.

Furthermore, since the bromide distribution ratio decreased in the acidosis-bromide experiments, it would at first glance appear to be necessary to assume that the hypothetical combination was not stable at low as well as high pH's. However, the production of the acidosis involved the elevation of the blood chlorides as well as the lowering of the pH, therefore, it is possible that the hypothetical reaction $XBr + Cl \rightleftharpoons XCl + Br$ was pushed to the right which would result in a lower bromide distribution ratio without the necessity of postulating a pH effect on the stability of XBr.

The Gibbs-Donnan Distribution Law, applied to semipermeable membranes, is based on the Second Law of Thermodynamics and is a necessary description of distribution at equilibrium providing one is dealing with activities and not with molecular concentrations. When, however, cells and serum are analyzed for bromide and chloride, molecular concentrations and not ion activities are determined. The discrepancies between the bromide concentrations as actually found and as predicted by the Gibbs-Donnan Law indicate that some unknown factor affects the free activity of the ions. In other words, there is a large discrepancy between the concentrations of the bromides and their ion activities. It was the main object of this work to determine whether the pH of the blood may be the determining factor, that is responsible for this discrepancy. The results of this work show that the reaction of the blood does have some influence on the bromide distribution between cells and serum, but the interpretation of this effect is difficult, since both alkalosis and acidosis tend to diminish the bromide cells to serum ratio. At present, therefore, the question must be left with a mere statement of the facts.

Conclusions

On the basis of the foregoing considerations the main conclusions of this study may be restated briefly as follows:

1. Sodium bicarbonate decreased the distribution ratio of bromide between cells and serum, but it delays the removal of ingested bromide from the blood.

2. Ammonium chloride also decreases the distribution ratio of bromide between cells and serum but it accelerates the removal of ingested bromide from the blood.

PART II.

INTRODUCTION

The second part of this study deals with the concentration and distribution of bromide and chloride in human subjects, first, between blood cells and serum, second, between blood serum and cerebrospinal fluid.

The first problem is related to the work of Hastings, Sendroy, McIntosh and Van Slyke (9) whose studies were undertaken in order to determine whether the Gibbs-Donnan Law accounts for the distribution of diffusible ions in the blood of human subjects. These authors concluded that chloride and bicarbonate ions were distributed in the blood of normal and pathological subjects in a manner consistent with that predicted by the Gibbs-Donnan Law. In view of the results for dogs reported in Part I, it was thought advisable to examine the distribution of bromides and chlorides in the blood of humans to whom sodium bromide had been administered. This constitutes the first problem.

The second problem is a part of the larger problem of the distribution of diffusible ions between blood serum and body fluids in general. The distribution of diffusible ions between blood serum and edema fluid has been studied by various workers who have shown the applicability of the Gibbs-Donnan Law to such systems. Studies, however, of the distribution of electrolytes between blood serum and cerebrospinal fluid have shown instances of inconsistency between the observed and predicted distributions. These observations led to the study of the distribution of bromides and chlorides between blood serum and cerebrospinal fluid. This constitutes the second problem.

METHOD OF PROCEDURE

The two problems outlined in the introduction were studied in two series of experiments. The first series consisted of seventeen determinations of bromide, chloride and water content of the cells and serum of eleven human subjects. The second series consisted of ten determinations of bromide and chloride of the serum and cerebrospinal fluid of eight human subjects.

The method of procedure was in general the same as that used in Part I, but in addition to cells and serum, cerebrospinal fluid was also analyzed by the same method as serum but expressed as millimols per liter of fluid. Bromides of serum and cerebrospinal fluid were also determined colorimetrically. There was also a difference in the mode of administration of sodium bromide. These differences will be described in the following sections.

Administration of Sodium Bromide

In the experiments of Part I a single large dose of sodium bromide was administered orally; in the experiments of this part the administration was in repeated small doses. The subjects of the experiments consisted of two groups, the first which had already received sodium bromide for therapeutic purposes, the second to which sodium bromide was administered for the purpose of bromide and chloride studies.

Two cases of the first group deserve special description. One subject, Ha, had received repeatedly 5 gram doses of sodium bromide intravenously, and samples of blood were drawn at various periods following the injections. Another subject, Ol, had bromide poisoning following intensive sodium bromide treatment; sodium chloride was administered to displace the bromide and samples of blood were drawn one before and one after the sodium chloride administration.

Sodium bromide was administered according to Hauptman's modification of the Walter bromide method (11) to the second group consisting of paretic and non-paretic subjects. The subject was given 0.02 gram of sodium bromide per kilogram of body weight, three times a day for five days. Twenty hours following the last dose of bromide samples of blood and cerebrospinal fluid were withdrawn.

Determination of Bromide Colorimetrically

The colorimetric determination of bromide in serum and cerebrospinal fluid was made according to Hauptman's modification of the Walter bromide method already referred to. The diluted serum (1 to 3) and the undiluted cerebrospinal fluid were deproteinized with trichloroacetic acid. The protein free filtrates were treated with gold chloride and compared in a Duboscq colorimeter with a standard bromide solution.

EXPERIMENTAL RESULTS

Halides in Blood Cells and Serum

The results of the first series of experiments are shown in Table X. It includes the following data:

1. The concentration of bromide, chloride and total halide in the cells and serum.
2. The percentage of total halide present as bromide in the cells and serum.
3. The distribution of bromide, chloride and total halide in the cells and serum.

Concentration.- Table X shows that in fifteen of the seventeen cases examined the bromides were more concentrated in the cells than in the serum and the difference was greater than that observed in dogs. The chlorides, on the other hand, were more concentrated, as is normally the case, in the serum than in the cells. In human subjects the total halide was not influenced as in the dog by the quantity of bromide present and it did not vary from subject to subject but approximated 110 millimols in all cases.

Percentage.- Table X shows that in twelve of the thirteen cases examined the percentage of total halide present as bromide in the cells was greater than that in the serum. This difference was found to apply also to dogs but the difference for humans was much greater than that for dogs.

Distribution.- Table X also shows that in nine of the thirteen cases examined the total halide cells to serum ratio was slightly above the numerical value of 0.72 usually encountered in the chloride cells to serum ratio of normal subjects. The chloride cells to serum ratio, on the other hand, was lower than 0.72 in ten out of thirteen cases. Similar results were obtained in the experiments on dogs. The bromide cells to serum ratio was considerably higher than 0.72 in sixteen out of seventeen cases. The ratio varied from 0.9 to 2.95, the variability not depending wholly on the mode of administration, the time interval between the administration and the blood sampling or the quantity of bromide present. The average of these ratios was much higher for the human subjects than for the dogs (Figure 14 A).

TABLE X
HALIDE DISTRIBUTION BETWEEN BLOOD CELLS AND SERUM OF HUMAN SUBJECTS
FOLLOWING SODIUM BROMIDE ADMINISTRATION

Subject	Time Since NaBr Admin- istration	(Br) ^s	(Br) ^o	$\frac{(Br)^o}{(Br)^s}$	(Cl) ^s	(Cl) ^o	$\frac{(Cl)^o}{(Cl)^s}$	$\frac{(Br)^s}{(Br)^s + (Cl)^s}$	$\frac{(Br)^o + (Cl)^o}{(Br)^s + (Cl)^s}$	$\frac{(Br)^s}{(Br)^s + (Cl)^s}$	$\frac{(Br)^o}{(Br)^o + (Cl)^o}$	H ₂ O	Notes	
S.M.	20	8.41	17.4	2.07	102.6	79.4	0.77	111.0	96.8	0.87	0.076	0.180	Wal- ter Meth- od	
L.E.	20	6.36	15.9	2.50	105.4	74.3	0.705	111.8	90.2	.807	0.057	.176	"	
G.O.	20	5.2	12.2	2.35	-	67.1	-	-	79.3	-	-	.154	"	
F.R.	20	6.48	14.6	2.25	105.3	70.6	0.671	111.8	85.2	0.680	0.058	.171	"	
P.H.		13.74	14.44	1.05	92.26	61.5	.677	106.0	75.9	.716	.130	.190	Treat- ment	
O.L.		36.4	39.35	1.08	71.0	39.65	.559	107.4	79.0	.735	.340	.498	"	
O.L.		8.95	13.47	1.51	101.1	69.1	.683	110.0	82.6	.751	.081	.163	NaCl Treat- ment	
R.O.		5.34	6.65	1.24	106.5	73.2	.688	111.8	79.9	.715	.048	.083	"	
C.O.	120	8.0	23.6	2.95	105.0	57.4	.547	113.0	81.0	.717	.071	.291	.677	"
R.I.	6	24.8	28.2	1.14	-	-	-	-	-	-	-	-	"	"
R.I.	168	19.6	18.0	0.918	93.4	-	-	113.0	-	-	-	-	"	"
H.A.	0.25	11.83	12.34	1.04	97.4	68.9	0.707	109.2	81.2	0.744	0.108	0.152	Intra- venous	
												0.925	0.690	

TABLE X
(Continued)

HALIDE DISTRIBUTION BETWEEN BLOOD CELLS AND SERUM OF HUMAN SUBJECTS

FOLLOWING SODIUM BROMIDE ADMINISTRATION

Subject	Time Since NaBr Admin- istration	(Br) _s	(Br) _c	$\frac{(\text{Br})_c}{(\text{Br})_s}$	(Cl) _s	(Cl) _c	$\frac{(\text{Cl})_c}{(\text{Cl})_s}$	$\frac{(\text{Br})_s}{(\text{Br})_c + (\text{Cl})_s}$	$\frac{(\text{Br})_c + (\text{Cl})_c}{(\text{Br})_s + (\text{Cl})_s}$	$\frac{(\text{Br})_c}{(\text{Br})_c + (\text{Cl})_c}$	H ₂ O		Notes
H.A.	96	9.01	5.79	0.64	95.4	75.3	0.790	104.4	81.2	0.777	0.928	0.674	Intra- venous
H.A.	0.25	12.2	23.68	1.94	95.0	57.3	.603	107.2	81.0	.755	.928	.674	"
H.A.	120	7.52	18.4	2.405	99.6	63.3	.635	107.1	81.7	.763	.926	.675	"
H.A.	0.25	7.0	7.85	1.121	-	-	-	-	-	-	-	-	"
E.M.	-	7.55	12.8	1.7	103.4	73.4	.710	111.0	86.2	.777	-	-	Treat- ment

Halides in Blood Serum and Cerebrospinal Fluid

The results of the second series of experiments on paretic and non-paretic subjects are shown in Table XI. It includes the following data:

1. The concentration of bromide, chloride and total halide in the blood serum and cerebrospinal fluid.
2. The percentage of total halide present as bromide in the blood serum and cerebrospinal fluid.
3. The distribution of bromide, chloride and total halide between blood serum and cerebrospinal fluid.
4. A comparison of the bromide blood serum to cerebrospinal fluid ratios as obtained electrometrically and colorimetrically.

Concentration.- Table XI shows that the chloride and the total halide concentrations of the cerebrospinal fluid were invariably greater than those of the serum. These results are in accord with the relationships normally existing in blood serum and cerebrospinal fluid. On the other hand, the bromide concentrations of the cerebrospinal fluid were in most cases smaller than those of the serum. Corresponding relationships were found between the halide concentrations of serum and cells both of dogs and of human subjects.

Percentage.- Table XI shows that in most cases the percentage of total halide present as bromide was greater in the serum than in the cerebrospinal fluid. A corresponding relationship was found between cells and serum both of dogs and of human subjects.

Distribution.- Table XI also shows that invariably the total halide serum to cerebrospinal fluid ratio approximates 0.88, the value normally encountered in the chloride serum to cerebrospinal fluid ratio of normal subjects. The chloride serum to cerebrospinal fluid ratio was in most cases lower than 0.88. On the other hand, the bromide serum to cerebrospinal fluid ratio was higher than 0.88 in seven out of eight cases. Almost corresponding distributions were found between the halides of cells and serum both of dogs and of human subjects.

Comparison.- Table XI shows that the bromide serum to cerebrospinal fluid ratios as determined colorimetrically are generally higher than those obtained electrometrically. The electrometric values fell chiefly between 1.4 and 2.0 while the colorimetric values fell between 2.5 and 3.1. By neither method could consistent differences be found between paretic and non-paretic subjects.

TABLE XI

HALIDE DISTRIBUTION BETWEEN BLOOD SERUM AND CEREBROSPINAL
FLUID FOLLOWING SODIUM BROMIDE ADMINISTRATION

Subject	Electrometric Method										Walter Method				Diagnosis
	$\frac{\text{mM}}{\text{K}^+\text{H}_2\text{O}}$ (Br) ^s	$\frac{\text{mM}}{\text{Na}^+\text{Cl}}$ (Br) ^{ost}	$\frac{(\text{Br})^s}{(\text{Br})^s + (\text{Br})^{\text{ost}}}$	$\frac{\text{mM}}{\text{K}^+\text{H}_2\text{O}}$ (Cl) ^s	$\frac{\text{mM}}{\text{K}^+\text{H}_2\text{O}}$ (Cl) ^{ost}	$\frac{(\text{Cl})^{\text{ost}}}{(\text{Cl})^s + (\text{Cl})^{\text{ost}}}$	$\frac{(\text{Br})^s}{(\text{Br})^s + (\text{Br})^{\text{ost}}}$	$\frac{\text{mM}}{\text{K}^+\text{H}_2\text{O}}$ (Br) ^{ost} + (Cl) ^{ost}	$\frac{(\text{Br})^s + (\text{Cl})^s}{(\text{Br})^s + (\text{Cl})^s + (\text{Br})^{\text{ost}} + (\text{Cl})^{\text{ost}}}$	$\frac{(\text{Br})^{\text{ost}}}{(\text{Br})^{\text{ost}} + (\text{Cl})^{\text{ost}}}$	$\frac{(\text{Br})^s}{(\text{Br})^s + (\text{Cl})^s}$	$\frac{(\text{Br})^{\text{ost}}}{(\text{Br})^{\text{ost}} + (\text{Cl})^{\text{ost}}}$	$\frac{(\text{Br})^s}{(\text{Br})^s + (\text{Cl})^s}$	$\frac{(\text{Br})^{\text{ost}}}{(\text{Br})^{\text{ost}} + (\text{Cl})^{\text{ost}}}$	
L.I.	-	-	-	-	-	-	-	-	-	-	2.86	-	2.86	-	Paresis
L.I.	4.8	3.4	1.41	106.7	124.5	0.857	111.5	128.3	0.869	0.030	0.043	0.030	2.50	0.030	"
S.M.	8.4	2.4	3.5	102.6	132.1	.777	111.0	134.5	.826	.018	.076	.018	2.72	.018	"
L.E.	6.4	3.24	1.96	105.4	124.0	-	111.8	127.2	-	-	.057	-	2.50	-	"
G.O.	5.2	3.3	1.56	-	-	-	-	-	-	-	-	-	2.80	-	Infectious titis
F.R.	8.5	3.3	1.99	103.3	122.9	0.841	111.8	126.2	0.886	0.076	0.076	0.026	3.1	0.026	Eczema
O.L.	36.4	19.0	1.91	71.0	105.0	.677	107.4	124.0	.884	.339	.339	.153	-	.153	Duodenal ulcer
O.L.	8.95	14.9	0.60	101.0	108.1	.935	110.0	123.0	.894	.082	.082	.121	-	.121	"
R.O.	5.38	6.65	.81	-	-	-	-	-	-	-	-	-	-	-	Neurasthenia
R.I.	24.8	12.14	2.04	-	-	-	-	-	-	-	-	-	-	-	Epilepsy

DISCUSSION AND CONCLUSIONS

Discussion

The experimental results will be discussed under the following headings:

1. The effect of sodium ion diffusibility on the total halide ion distribution.
2. The applicability of the Gibbs-Donnan Law to the distribution of bromide ions between cells and serum and between serum and cerebrospinal fluid.
3. The validity of the Malamud bromide permeability index in paresis.

Sodium Ion Diffusibility Effect

The administration of sodium bromide to a normal dog or human subject resulted in a total halide cells to serum distribution ratio higher than the chloride ratio before administration. This rise can be adequately explained by the indiffusibility of the sodium ion through the cell membrane, as was explained in Part I. On the other hand, the distribution ratio of total halide between serum and cerebrospinal fluid after the administration of sodium bromide was approximately the same as that of the chloride before administration. This absence of difference can be explained by the diffusibility of the sodium ion through the barrier separating blood serum and cerebrospinal fluid.

Applicability of the Gibbs-Donnan Law

The bromide distribution ratios between cells and serum and between serum and cerebrospinal fluid of human subjects were greater than those which might be predicted by the Gibbs-Donnan Law. The discrepancy between observed and predicted ratios was even greater in human subjects than in dogs. Other evidence exists which points to the absence of complete freedom of passage of small molecules and even ions from serum and cerebrospinal fluid. It is not surprising, therefore, that bromides are found in higher concentration in the serum than in cerebrospinal fluid. This provides another example of the inadequacy of the Gibbs-Donnan Law to

account for the unequal distribution of ions and emphasizes the necessity of applying it only when thermodynamic equilibrium can be demonstrated.

Validity of the Bromide Permeability Index in Paresis

A theory was advanced by Malamud, Fuchs and Malamud (12) that the barrier between serum and cerebrospinal fluid was such that the bromide distribution ratio between serum and cerebrospinal fluid of the non-paretic individual has a value of 3 to 1, whereas in the paretic this ratio decreases and according to these authors indicates a decrease in the resistance of the barrier. The 3 to 1 ratio was obtained by these authors with the Walter colorimetric method. Bromide distribution ratios were determined in the second series of experiments reported in this part both by the electrometric and the Walter colorimetric method. The electrometrically determined ratios for both paretic and non-paretic individuals ranged mostly between 1.4 and 2.0 which are in close agreement with the range 1.48 to 1.76 reported by Dalma (13) in a series of dialysis experiments. The colorimetrically determined ratios ranged from 2.5 to 3.1, the greater uniformity as compared with the electrometric method being probably due to lesser sensitivity of the colorimetric method. Neither method showed any discrimination between paretic and non-paretic individuals. These results are based on too few cases to warrant a definite conclusion, but they indicate the need of further work before the bromide permeability index should be used for diagnostic purposes.

Conclusions

On the basis of the discussion in the preceding section the main conclusions of this study may be briefly stated as follows:

1. The Gibbs-Donnan Distribution Law does not explain the bromide distribution ratio between cells and serum, and between serum and cerebrospinal fluid of human subjects.
2. The bromide distribution ratios between serum and cerebrospinal fluid of paretics and non-paretics are not sufficiently differentiated to permit their use for diagnosis.

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